PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

To:

see form PCT/ISA/220

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Applicant's or agent's file reference see form PCT/ISA/220	Date of mailing *(day/month/year)* see form PCT/ISA/210 (second sheet) **FOR FURTHER ACTION** See paragraph 2 below

International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/BE2005/000017	07.02.2005	06.02.2004

International Patent Classification (IPC) or both national classification and IPC
G01N33/50, G01N33/573

Applicant
UNIVERSITE DE LIEGE

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☒ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will usually be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA"). However, this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notifed the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of three months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA:

European Patent Office
D-80298 Munich
Tel. +49 89 2399 - 0 Tx: 523656 epmu d
Fax: +49 89 2399 - 4465

Authorized Officer

GONCALVES MAUGER, M

Telephone No. +49 89 2399-8127

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

International application No.
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Box No. I Basis of the opinion

1. With regard to the **language**, this opinion has been established on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ This opinion has been established on the basis of a translation from the original language into the following language , which is the language of a translation furnished for the purposes of international search (under Rules 12.3 and 23.1(b)).

2. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application and necessary to the claimed invention, this opinion has been established on the basis of:

   a. type of material:

   ☐ a sequence listing

   ☐ table(s) related to the sequence listing

   b. format of material:

   ☐ in written format

   ☐ in computer readable form

   c. time of filing/furnishing:

   ☐ contained in the international application as filed.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority for the purposes of search.

3. ☐ In addition, in the case that more than one version or copy of a sequence listing and/or table relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

4. Additional comments:

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

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Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application,

☒ claims Nos. 10

because:

☐ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination *(specify)*:

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. are so unclear that no meaningful opinion could be formed *(specify)*:

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for the whole application or for said claims Nos. 10

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form	☐	has not been furnished
	☐	does not comply with the standard
the computer readable form	☐	has not been furnished
	☐	does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See separate sheet for further details

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

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Box No. IV Lack of unity of invention

1. ☒ In response to the invitation (Form PCT/ISA/206) to pay additional fees, the applicant has:

   ☐ paid additional fees.

   ☐ paid additional fees under protest.

   ☒ not paid additional fees.

2. ☐ This Authority found that the requirement of unity of invention is not complied with and chose not to invite the applicant to pay additional fees.

3. This Authority considers that the requirement of unity of invention in accordance with Rule 13.1, 13.2 and 13.3 is

   ☐ complied with

   ☒ not complied with for the following reasons:

   see separate sheet

4. Consequently, this report has been established in respect of the following parts of the international application:

   ☐ all parts.

   ☒ the parts relating to claims Nos. 1-9, 11-14

Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-9, 11-14
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-9, 11-14
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1(?)-9, 11-14
	No:	Claims	

2. Citations and explanations

   see separate sheet

1. The application lacks unity within the meaning of Rule 13.1 PCT. The following separate inventions or groups of inventions are not so linked as to form a single general inventive concept:

1 ...Claims 1-9 and 11-14: Methods and kits for in vitro measurement of neutrophil cell activation ans screening methods for inhibitors of myeloperoxidase, through the accurate detection of myeloperoxidase levels.

2 ...Claim 10: The use of nitrite to enhance the enzymatic determination of a peroxidase.

The Sets of claims 1 and 2 have in common that they involve measuring a peroxidase.

Methods and devices for the enzymatic measurement of peroxidases are however known, for example, from document D1 (WO9961907, cited in the application, see claims). Thus these common features of the claims D1 not provide a single general inventive concept linking the claims.

The subject-matter of claims 1-9 and 11-14 in essence differs from that known from document D1 in that only content in myeloperoxidase is measured. The problem addressed in these claims im view of document D1 is to provide a **method which is more accurately able to measure myeloperoxidase activity** in complex biologicalsamples (without the interference of other peroxidases). This problem is addressed by capturing the MPO present in the biological sample (via an MPO specific antibody), before an appropriate selection of assays for MPO detection.

The subject-matter of claim 10 in essence differs from that known from document D1in that peroxidase activity is measured by use of a nitrite in addition to the hydrogen peroxide and a chromogen. The problem addressed in the claim 10 im view of document D1 is the improvement of the enzymatic chromogenic measurement of peroxidases. This problem is addressed by adding nitrite to the reaction medium to enhance the enzymatic reaction.

The above analysis demonstrates that the subject-matter of the two groups of claims is also not linked by providing a solution to a common problem.

In conclusion neither the technical features in common to the groups of claims nor the problem solved by each of the two groups of claims provides a corresponding special technical feature, which establishes a single general inventive concept linking any of the two sets of claims.

Thus the technical relationship between the subject-matter of the sets of claims is lacking, and the requirement for unity of invention referred to in Rule 13.1 PCT is not fulfilled.

2. The following documents are mentioned in the search report:
D1: WO 99/61907,
D2: EQUINE VETERINARY JOURNAL, vol. 32, no. 4, July 2000, pages 327-333,
D3: EQUINE VETERINARY JOURNAL, vol. 31, no. 4, July 1999, pages 331-335,
D4: CANADIAN JOURNAL OF VETERINARY RESEARCH, vol. 63, no. 2, April 1999, pages 142-147,
D5: AMERICAN JOURNAL OF VETERINARY RESEARCH, vol. 60, no. 7, July 1999, pages 807-813,
D6: AMERICAN JOURNAL OF VETERINARY RESEARCH, vol. 55, no. 10, 1994, pages 1454-1463,
D7: CANADIAN JOURNAL OF VETERINARY RESEARCH, vol. 62, no. 2, April 1998, pages 127-132,
D8: WO 02/50550,
D9: VETERINARY IMMUNOLOGY AND IMMUNOPATHOLOGY, vol. 66, no. 3-4, 11 December 1998, pages 257-271.

3. The subject-matter of claim 1 is a method for in vitro measurement of neutrophil cell activation through the specific measurement of myeloperoxidase content only. Such a method is not anticipated by the disclosures in the known prior art documents and is thus novel (Article 33 (2) PCT).

4. The method of claim 1 cannot be derived from the known prior art documents, either taken alone or in combination.
The closest prior art is considered to result from D1. This document describes a method

for the measurement of the activation status of leucocyte cells, obtained by the measurement of the total peroxidase activity of said leucocyte cells. The leucocyte cells can be for example eosinophils, neutrophils and other blood cell types(see claims).

The method of claim 1 is limited to myeloperoxidase activity per se, instead of all kinds of peroxidase activity in D1. The method of D1 also does not apply to complex a cellular media such as plasma. The method of claim 1 provides specific measurement of neutrophil cell activation in a biological sample, by the specific measurement of myeloperoxidase in complex cellular or acellular biological media.
The problem to be solved by the method of claim 1 is thus to provide a method which is **more accurately able to measure myeloperoxidase activity** (and neutrophil activation) in complex biological samples .
The method of claim 1 solves the problem, by capturing the myeloperoxidase that is present in the biological sample by specific antibodies, before an appropriate selection of assays for MPO detection.
The subject-matter of claim 1 also can characterize total (active and non-active) myeloperoxidase, or just the active myeloperoxidase obtained from the neutrophil cells. Due to this additional unexpected effect, the presence of an inventive step can be acknowledged (Article 33 (3) PCT).

5. Dependent claims numbers 2 to 6 add features to the method of claim 1 and thus also relate to novel and inventive subject-matter (Article 33 (2) and (3) PCT).

6. The above comments also apply to the subject-matter of independent claims 7 and 8, referring to kits specially adapted for the performance of the method of claim 1 either by ELISA or SIEFED; to claims 9 and 11, referring to uses of the method of claim 1; to claim 12 , referring to a method of screening for inhibitors of myeloperoxidase that comprises the capturing of the myeloperoxidase as first step; and to the claims dependent thereon (Article 33 (2) and (3) PCT) .

7. For the sake of clarity and to fulfil the requirements for patentability, the step of "obtaining a biological sample" should be deleted from the wording of claim 1.